Genetic Diversity among World Hop Accessions Grown in the USA

J. A. Henning,* J. J. Steiner, and K. E. Hummer

ABSTRACT

Hop (*Humulus lupulus* L.) is an important cash crop in the U.S. Pacific Northwest. Classifying groups of hop accessions presently held in the USDA-ARS world collection is vital toward categorizing newly imported accessions and identifying closely related (if not identical) cultivars. The objective of this study was to identify hop germplasm diversity pools on the basis on morphological and chemical data by cluster analysis. Eight hop quality characteristics including yield (YLD), α acids, β acids, hop-storage index (HSI), cohumulone (CoH), myrcene (M), caryophyllene (C), and humulene (H) were obtained from historical databases for 129 accessions from the USDA-ARS hop germplasm field collection located near Corvallis, OR. Three distinct genetic diversity pools were identified and named: (i) European, (ii) Wild North American, and (iii) Hybrids. The European pool was divided into English and Continental European subgroups distinguished by their α-acids and CoH contents. The Hybrid pool was divided into five subgroups distinguished by their geographic origins. The variables YLD and CoH content differentiated these five subgroups ($r = 0.92$; $P \leq 0.05$). The information presented in our study will help categorize newly imported accessions into the current U.S. hop germplasm collection and will help in identifying closely related or similar accessions.

Hop is a dioecious climbing plant with bines that twine in a clockwise direction. Plants typically grow on 6-m trellises with the mature female floral structure, called hop cones (or strobiles), as the harvested portion of the plant. Lupulin glands located on the bracteoles, and to a lesser extent on the bracts, is the source of commercial value in hops. Resins within these glands give beer its bitterness while the essential oils found in the glands contribute flavoring. Hop cones were initially utilized as a preservative in beer brewing. Later, after the advent of pasteurization, hop cones were used as flavoring agents as people began to associate hop flavor with beer.

Most early hop used for beer production in the USA was imported from European countries, including Germany and England. Cultivars such as Fuggle, Saazer, Bullion, and Halletauer Mittelfruh were subsequently introduced into the USA for production as opposed to importing hop cones for brewing. Since that time, numerous foreign-developed cultivars have found their place in the USA hop brewing industry, and production of these cultivars continues in many cases. At the same time, many new domestic-developed hop cultivars have been released and collections of wild hop accessions have been pursued both within the USA and other countries. The end result of all this activity is a large holding of hop accessions with little descriptive information about their relationships.

One method of identifying similar accessions and assessing genetic and phenotypic relatedness is to perform a classification on a large collection of individuals using a statistical procedure such as cluster analysis (Anderberg, 1973). Multiple characters for each individual are used to group accessions into cluster classes. Individuals within a given cluster class are similar, while individuals from different classes are not. Similarity measurements among clusters were also determined so that relationships between groups can be established. Use of classification data can offer the hop breeder an objective judgment when determining which widely differentiated individuals to use as parents. It can also be used to classify newly introduced accessions into known population groups and determine similarity or novelty with existing collection holdings.

Several papers have discussed hop genetic variation using either biochemical or DNA descriptors. In almost all cases, cluster analysis identified two primary groups: the so-called European and American populations. Sustar-Vozlic and Javornik (1999) analyzed 65 world hop cultivars using both random amplified polymorphic DNA (RAPDs) and dried hop cone essential oil composition. They observed the two primary groupings with further subdivision of the European group into five distinct clusters corresponding to regions of geographic adaptation. The authors stated that the RAPD data corresponded well with essential oil fingerprints groupings. Murakami (2000) also assayed 51 world cultivars using RAPD analysis and identified six clusters that were reported to agree with breeding history and country of origin, although some associations were not readily apparent. Seefelder et al. (2000) analyzed 84 world cultivars and six German experimental lines for genetic relatedness using amplified fragment length polymorphism (AFLP). Seven AFLP primer pair combinations produced a total of 130 polymorphic fragments that categorized two main clusters. These first represented European aroma-type hop accessions while the second consisted of lines developed via the incorporation of genes from wild American hop accessions into European cultivars. Further subgroups were observed in each primary cluster with the authors stating the resulting dendrogram agreed with pedigree data. Several accessions had identical fingerprints and were therefore indistinguishable from one another. Even though Seefelder et al. (2000) used German hop collection accessions, more than a third covered were not used for breeding or production in the USA, and no wild American accessions were included. Jakse et al. (2001) utilized AFLP techniques to differentiate European and American hop

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cultivars, but failed to do so using microsatellites. The AFLP could be used to produce several subsets clusters that agree with geographic groupings with one of these subclusters segregating out those cultivars that were aroma-type hybrids with the cultivar, Northern Brewer. Finally, Patzak (2001) compared four DNA techniques by biochemical fingerprints to estimate genetic relatedness. Three out of the four DNA techniques [RAPD, sequence tagged sites (STS), and inter-simple sequence repeat (ISSR)] failed to differentiate among three clonal selections from a Saazer population while the AFLP and biochemical data differentiated among the clonal selections. Correspondence analysis of the five techniques using cophenetic correlation coefficients demonstrated a high similarity among dendrograms estimated using DNA techniques ($r \geq 0.86$), but low correspondence between DNA techniques and biochemical characters ($r \leq 0.59$).

In the aforementioned studies using DNA markers, none included wild American germplasm. With the exception of Small's work (1978, 1980) using solely morphological characteristics, only one other published study (Stevens et al., 2000) utilized wild American hop accessions. It was found that two closely related flavonoids were distributed across wild North American accessions and also in some of the hybrid cultivars that resulted from crosses between wild accessions and European hop cultivars. These same two flavonoids were not observed in nonhybridized European cultivars. However, the use of these two flavonoids as characterization variables was not sufficient to differentiate subgroups within domesticated hybrids or European accessions.

From the standpoint of germplasm collection and new accession classification, the absence of information about wild North American germplasm is a major gap. Since DNA techniques are not always readily available to all research groups, studies using commonly accessed traits of economic importance would greatly benefit a broad spectrum of researchers. Finally, phenotypic information on economic traits allows collection population structures to be defined so breeders or growers can identify specific accessions that may be of interest to them. The objective of this study was to identify distinct pools of female hop genetic diversity on the basis of yield, hop-storage-index, α acids, β acids, cohumulone, myrcene, caryophyllene, and humulene contents.

MATERIALS AND METHODS

Plant Materials and Quality Evaluations

All plant materials were grown on the USDA-ARS Hop Research Facility located on the Oregon State University, Hyslop East Farm near Corvallis, OR. Data from 129 female accessions from Europe, North America, Japan, Australia, New Zealand, and South Africa were collected over a 25-yr period with replicated observations occurring over time. Two additional clones of the cultivar Early Prolific, grown on different plots, were included as controls. All data were standardized to a moisture content of 80 g kg^{-1}. Hop cone yield, bittering acids (α- and β-acid content), CoH, and HSI were reported as published by Henning et al. (1997). Levels of M, C, and H were reported as percentages of the total essential oil extract. All data were averaged across years and only entries with at least three replicate year's data were included.

Statistical Methods

Pearson's correlation coefficient (r) was used to describe associations among the eight hop quality descriptors. Probabilities for the significance of all correlation coefficients were determined by Bonferroni inequality adjustment (Snedecor and Cochran, 1980). The 129 accessions examined were grouped into genetic diversity pools by cluster analysis based on Euclidean distance and Ward's (1963) clustering technique (Systat for the Macintosh, SPSS, Chicago, IL) with all data transformed by the standard normal deviate (Snedecor and Cochran, 1980). The designated genetic diversity pool classes were determined by the optimal number of classes (c_{opt}) method (Steiner et al., 2001):

$$c_{opt} = \lim [D_n \geq 0.5 \bullet D_g]; \text{ whenever } n > 2$$

where D_g was the greatest amalgamation distance between two clusters and D_n was the least successive amalgamation distance between two clusters that was greater than or equal to one-half D_g. The significance of each of the eight quality descriptor in developing the three genetic diversity pools and the percentage of correctly classified accession cases were tested using Wilks' Lambda statistic by step-wise discriminant analysis (SPSS, Inc., Chicago, IL). The accession genetic diversity pool membership was used as the grouping variable. The maximal number of significant canonical discriminant functions needed to describe the core subset was also determined, and percentage of accessions that were correctly classified was determined.

Each hop quality descriptor was tested for differences within each genetic diversity pool on the basis of natural geographic groupings within the pools. Subgroups within the genetic diversity pools were recognized if two or more geographic subgroups had at least one significantly different hop quality descriptor as determined by analysis of variance. Interpretive group classes for each of the eight hop quality measures were assembled using cluster analysis based on Euclidean distance and Ward's (1963) clustering technique (Systat for the Macintosh, Evanston, IL) (Steiner et al., 2001). The number of categorical classes for each hop quality descriptor interpretive group was: yield (4 classes); α acids (5 classes); β acids (2 classes); HSI (4 classes); CoH (5 classes); M (4 classes); C (3 classes); and H (3 classes). The number of classes was based on a visual examination of the quality measure cluster analysis dendrograms. Class differences were verified by analysis of variance and Fisher's protected least significant difference test. Genetic diversity pools and geographic subgroups summary statistics included the mean, minimum, maximum, standard error of the mean, and mode for each interpretive group descriptor. A similarity index for interpretive group classes was calculated for each descriptor within each genetic diversity pool and significant geographic subgroups by the following equation:

$$I = \{S - [\sum_{k \in n} diff(x_{ik}, x_{jk}, .. x_{nk})]\}/S,$$

where the similarity index (I) for a set of observations with S possible comparisons for an interpretive group descriptor (k), for a genetic diversity pool or geographic subgroup with n accessions, and $x_{ik}, x_{jk}, .. x_{nk}$ being the k states of the descriptor in the pool or subgroup. The S possible combinations of interpretive group states for comparison in a genetic diversity pool were determined by:

$$S = \left[\frac{n!}{(n - r)!}\right]/2,$$

where n is the total number of accessions in a genetic diversity pool and $r = 2$ (two accessions compared at a time). When all x_{ik} states of k in a genetic diversity pool are the same, $I = 1.0$. Differences in the levels of diversity among subgroups were tested by analyzing differences in similarity index values for each subgroup. Friedman's nonparametric analysis of variance was used to test for significant differences among the eight-descriptor variables. We then tested specific comparisons among each subgroup by Wilcoxon's matched pairs test.

RESULTS AND DISCUSSION

We identified three distinct genetic diversity pools of the 129 accessions included in the study on the basis of cluster analysis with verification by discriminant analysis (Table 1). Discriminant analysis demonstrated that the greatest percentage of correctly placed accessions (96.9%) in the greatest number of groups was obtained with three genetic diversity pools. The three pools were described as European (EU), wild North American (WNA), and domesticated hybrids (HYB). Mean values for each descriptor variable differed among the three pools (Table 2). Mean values for yield, α acids, β acids, and *M* were highest for HYB, with intermediary values for CoH and H, and lowest values for HSI and C. These values are consistent with accessions that would typically be used for extract or bittering in beer brewing. Average values for EU were lowest for β acids, CoH, and M, with low but not significantly different from WNA for yield, α acids, and HSI. The EU group was highest for H and HSI, but not significantly different from WNA for this last HSI. These values are typical of accessions used primarily for aroma rather than bittering. The last group, WNA, exhibited the highest values for CoH and also exhibited high values (but not significantly different from one of the other groups) for HSI, M, and C. It had the lowest concentration of H while not differing from the EU population in yield, α, and β acids. No accessions from this population appear to be adequate for direct use in beer brewing since the required quality factors do not meet minimum standards.

Interpretive groups for each of the eight-descriptor characteristics were formulated and applied to the 129 accessions (Table 3). Most of the descriptive variables had enough range and distribution to include at least three and as many as five interpretive group classes for each trait. In addition, both the EU and HYB groups were split up into subgroups on the basis of geographic distribution (Table 3). The EU group was split into two subgroups: Continental Europe (CU) and United Kingdom (UK). The HYB group was divided into five subgroups: Continental Europe (CE-HYB), United Kingdom (UK-HYB), USA (USA-HYB), Asia (A-HYB), and former British Commonwealth countries (BC-HYB). We observed significant differences ($P \leq 0.05$) within primary groups and subgroups for levels of diversity as measured by the similarity indices. The UK-HYB group had the lowest average similarity index indicating the greatest amount of within-group variability, while the A-HYB and CE-HYB subgroups exhibited the highest average similarity index, thus exhibiting the least amount of within-group diversity. The diversity of the remaining WNA pool and subgroups were not significantly different from one another.

Table 1. Relationship between number of cluster analysis groups, cluster amalgamation distance between clusters, and the percentage of correctly placed accessions by discriminant analysis when using the number of cluster groups as the DA classification factors.

	Cluster analysis	
Number of groups	Amalgamation distance	Discriminant analysis accessions placement
	d	%
2	21.48	93.0
3	16.61	96.9
4	8.72	89.9
5	4.93	89.9
6	4.81	90.7
7	4.76	90.7
8	4.20	90.7

Specific relationships between each subgroup within a primary genetic diversity pool were tested to determine differences. Subgroups UK and CU differed in levels of α acid and cohumulone, with UK having higher levels of both chemicals (Table 4). This is most likely due to the British consumer's apparent preference for a more bitter beer than what American and Continental European consumers prefer. The subgroups in the HYB primary pool differed only in levels of cohumulone and in yield (Table 5). Not surprisingly, yields were highest in the USA-HYB subgroup (1296 kg ha^{-1}) and lowest in the CE-HYB (810 kg ha^{-1}). Cohumulone levels were highest for A-HYB (40.4%) and USA-HYB (36.6%), again reflecting the primary end-use preferences for most of the hops developed in both regions. Cohumulone levels were lowest in both CU and UK (26.3 and 29.2%, respectively). Whole or pellet hops are primarily used in these two regions because of brewery preference for low cohumulone. Variability in C levels observed among several subgroups of the HYB primary group may reflect European choice of hop cultivars with low levels of C while other regions, such as USA and former

Table 2. Means for three germplasm pools of hops [Domesticated hybrids (HYB), European (EU) and wild North American (WNA)] from a cluster analysis based on eight measures of quality: Yield, α-acid percentage, β-acid percentage, hop storage index (HSI), cohumulone percentage (CoH), Myrcene concentration (M), Caryophyllene concentration (C) and Humulene concentration (H).

	Group		
Variable	HYB	EU	WNA
Yield (kg ha^{-1})	1015.4a†	408.7b	507.8b
α acid (%)	8.3a	4.6b	3.6b
β acid (%)	4.7a	2.8c	3.4b
HSI	0.26b	0.28a	0.29a
CoH (%)	31.8b	25.3c	60.0a
M (v/v)	56.1a	38.1b	53.5a
C (v/v)	8.6b	9.8b	17.7a
H (v/v)	14.7b	25.6	5.4c

† Means within rows followed by a different letter are significantly different at $P \leq 0.05$ on the basis of Fisher's Protected Least Significant Difference test.

Table 3. Categorization of 129 USDA-ARS hop accessions into three genetic diversity pools on the basis of cluster analysis of eight quality descriptors. The quality descriptors are summarized as interpretive groups. Where subpool designations are reported, differences are based on significant differences for at least one quality descriptor by analysis of variance using the geographic groupings of accessions as the grouping.

				Interpretative group (IG) descriptors†							
Entry‡	Cultivar	Origin	Subpool	YLD (4)§	α Acids (5)	β Acids (2)	HSI (4)	CoH (5)	M (4)	C (3)	H (3)
				Genetic Diversity Pool							
				European							
21276	Early Prolific	UK	UK	4	4	1	2	5	3	3	2
21277	Early Promise	UK	UK	2	1	1	4	3	1	1	2
21284	Bramling	UK	UK	4	4	1	4	4	1	3	2
21396	Tolhurst	UK	UK	3	1	1	4	2	1	2	3
21668	White Golding	UK	UK	3	4	2	2	4	2	3	1
21680	East Kent Golding	UK	UK	4	4	2	3	4	4	1	1
21681	Canterbury Gold	UK	UK	4	4	2	3	4	4	1	1
48209	Fuggle H	UK	UK	2	4	2	2	4	4	2	1
66050	Alliance	UK	UK	4	4	1	2	1	1	2	3
66051	Progress	UK	UK	4	5	2	2	2	4	2	3
		Subpool IG mode:		4	4	1	2	4	1	2	1
		Similarity index¶:		0.49	0.58	0.55	0.47	0.33	0.40	0.44	0.13
21014	Mittelfrue	CU	CU	2	4	1	3	2	2	3	2
21045	Serebrianka	CU	CU	2	2	2	3	5	2	3	2
21049	Styrian	CU	CU	3	2	2	2	3	1	3	3
21077	Saazer	CU	CU	1	3	1	2	4	1	3	3
21079	Blue North Brewer	CU	CU	1	1	1	3	3	1	3	3
21114	Nadwislanska	CU	CU	4	4	2	2	5	3	3	1
21168	Precdbourg	CU	CU	2	4	2	4	4	3	3	1
21172	Landhopfen	CU	CU	2	3	1	3	2	1	3	3
21173	Strisselspalter	CU	CU	2	2	1	3	3	1	2	3
21197	USA Tettnang	UK	CU	3	3	1	2	3	1	2	2
21213	Aromat	CU	CU	3	3	1	3	5	3	3	2
21214	Sirem	CU	CU	4	3	1	3	4	3	3	2
21217	Star	CU	CU	1	2	1	3	3	1	2	2
21673	Hersbrucker Pure	CU	CU	4	4	2	1	5	4	3	1
61020	Savinja Golding	CU	CU	4	4	1	2	1	1	2	3
61021	Tettnanger	CU	CU	4	5	2	2	1	2	3	2
		Subpool IG mode:		2	4	1	3	3	1	3	2
		Similarity index:		0.49	0.58	0.55	0.47	0.33	0.40	0.44	0.13
				Wild North American							
21115	Pocket Talisman	USA-HYB	WNA	1	2	2	3	3	2	3	2
21563	Iowa 3	WNA	WNA	1	2	1	2	3	1	2	3
21565	Iowa 5	WNA	WNA	1	2	2	3	3	1	3	2
21566	Iowa 6	WNA	WNA	3	2	1	2	4	2	3	1
21567	Iowa 7	WNA	WNA	1	4	2	3	2	1	2	3
21568	North Dakota 1	WNA	WNA	2	4	2	3	3	1	2	2
21576	Montana 4	WNA	WNA	3	4	2	2	3	2	2	2
21581	Montana 9	WNA	WNA	2	3	1	2	3	1	2	2
21585	Montana 11	WNA	WNA	2	4	1	3	3	1	3	3
21590	Montana 16	WNA	WNA	2	3	2	3	3	1	3	3
21594	Montana 20	WNA	WNA	4	4	1	3	5	4	2	1
21596	Utah 11	WNA	WNA	4	5	2	2	5	4	3	1
21599	Utah 12	WNA	WNA	2	4	2	1	4	3	3	1
21600	Utah 13	WNA	WNA	4	5	2	2	5	4	3	1
21602	Montana 24	WNA	WNA	4	4	2	1	4	4	3	1
21605	W AM Minn.	WNA	WNA	4	5	2	3	5	4	3	1
60016	New Mexico 1-3	WNA	WNA	4	5	2	1	1	4	1	3
60027	Colorado 2-2	WNA	WNA	4	5	2	3	1	4	1	3
60029	Colorado 3-1	WNA	WNA	3	5	2	1	1	3	1	3
60032	Colorado 5-1	WNA	WNA	3	5	1	1	1	2	3	3
60033	Colorado 6-1	WNA	WNA	4	5	1	2	1	4	2	2
60035	Colorado 7-2	WNA	WNA	4	4	1	3	1	1	1	3
60038	Wyoming 3-1	WNA	WNA	4	4	2	3	1	1	1	3
66052	Pride of Ringwold	BC-HYB	WNA	3	5	2	2	1	3	1	3
		Pool IG mode:		4	4	2	3	3	1	3	3
		Similarity index:		0.32	0.34	0.55	0.39	0.30	0.31	0.38	0.37
				Hybrids							
19001	Brewers Gold	UK	UK-HYB	1	2	1	2	3	1	3	2
19120	Sunshine-S	UK	UK-HYB	2	3	2	2	4	3	3	3
21043	Challenger	UK	UK-HYB	3	3	1	3	5	2	2	1
21044	Northdown	UK	UK-HYB	1	2	1	2	5	1	3	3
21112	Target	UK	UK-HYB	1	1	1	2	3	2	2	3
21278	Keyworths Early	UK	UK-HYB	3	4	2	4	5	3	3	1
21280	Pride of Kent	UK	UK-HYB	4	3	2	3	4	2	2	2
21282	Saxon	UK	UK-HYB	2	1	1	4	4	1	2	2
21283	Viking	UK	UK-HYB	4	3	1	4	5	1	3	2
21498	Yeoman	UK	UK-HYB	1	4	1	3	3	1	3	2
21667	Omega	UK	UK-HYB	3	4	2	2	4	3	2	1
64100	Bullion	UK	UK-HYB	4	5	2	1	1	3	2	3
64107	Northern Brewer	UK	UK-HYB	4	4	1	2	1	1	3	3

Continued next page.

Table 3. Continued.

				Interpretative group (IG) descriptors†							
Entry‡	Cultivar	Origin	Subpool	YLD (4)§	α Acids (5)	β Acids (2)	HSI (4)	CoH (5)	M (4)	C (3)	H (3)
		Subpool IG mode:		1	3	1	2	4	1	3	2
		Similarity index:		0.21	0.18	0.49	0.27	0.21	0.31	0.46	0.29
21522	Saazer 36	CU	CU-HYB	2	4	1	4	2	2	2	2
21050	Ahil	CU	CU-HYB	2	2	1	3	5	3	3	1
21051	Apolon	CU	CU-HYB	2	1	1	2	2	2	3	3
21052	Atlas	CU	CU-HYB	2	3	1	3	4	3	3	1
21053	Aurora	CU	CU-HYB	1	3	2	2	4	2	3	1
21078	Record	CU	CU-HYB	2	3	1	2	4	2	3	3
21081	Dunav	CU	CU-HYB	4	4	1	2	5	3	3	1
21082	Neoplanta	CU	CU-HYB	1	4	1	4	5	3	3	2
21083	Vojvodina	CU	CU-HYB	2	4	1	3	5	3	3	1
21907	Huller Bitter	CU	CU-HYB	1	1	1	4	3	3	3	1
21169	Tardif de Bourg.	CU	CU-HYB	1	1	1	3	4	2	3	1
21170	Elsasser	CU	CU-HYB	4	3	2	2	4	3	2	1
21185	Hersbrucker	CU	CU-HYB	2	4	1	4	4	2	3	1
21186	Spalter	CU	CU-HYB	2	2	1	2	3	1	3	3
21187	Southern Brewer	BC	CU-HYB	2	2	1	3	3	2	3	1
21215	Norgard	CU	CU-HYB	1	4	1	3	2	3	2	2
21227	Perle	CU	CU-HYB	2	4	1	3	5	2	3	1
21239	Bobek	CU	CU-HYB	2	4	1	3	5	3	1	2
21496	Tettnanger A	CU	CU-HYB	2	2	1	4	4	4	3	1
21497	Tettnanger B	CU	CU-HYB	4	5	1	4	5	3	3	1
21518	Hersbrucker Alpha	CU	CU-HYB	2	4	1	3	3	2	2	2
21611	Celeia	CU	CU-HYB	4	5	2	4	5	4	3	1
21670	Magnum	CU	CU-HYB	4	5	2	1	5	3	3	1
21671	Hallertauer Gold	CU	CU-HYB	4	5	2	2	5	4	3	1
21672	Hall. Tradition	CU	CU-HYB	4	5	2	1	5	4	2	1
21674	Spalter Select	CU	CU-HYB	4	4	2	2	4	3	3	1
21675	Orion	CU	CU-HYB	4	4	2	2	4	3	3	1
21682	Wurttemberger	CU	CU-HYB	3	4	2	2	4	3	3	1
56002	Backa	CU	CU-HYB	2	4	2	3	4	4	2	
		Subpool IG mode:		2	4	1	3	4	3	3	1
		Similarity index:		0.32	0.27	0.54	0.27	0.30	0.34	0.62	0.52
21040	Columbia	USA	USA-HYB	2	4	2	2	3	3	3	1
21041	Willamette	USA	USA-HYB	2	3	1	2	5	2	3	1
21055		USA	USA-HYB	2	3	2	3	3	3	3	1
21182	Galena	USA	USA-HYB	2	1	1	3	3	3	2	2
21183	Eroica	USA	USA-HYB	3	2	2	2	4	3	2	1
21193	Nugget	USA	USA-HYB	3	3	2	2	3	3	3	1
21222	Aquila	USA	USA-HYB	1	3	1	2	3	1	3	3
21225	Olympic	USA	USA-HYB	2	2	1	3	3	1	1	2
21226	Chinook	USA	USA-HYB	2	3	1	4	5	3	2	1
21231	Pat Leavy Seed	USA	USA-HYB	4	4	1	2	5	3	3	1
21287	Banner	USA	USA-HYB	4	3	1	4	3	3	1	1
21455	Mt. Hood	USA	USA-HYB	2	4	2	4	4	1	3	2
21490	Crystal	USA	USA-HYB	4	4	2	3	3	3	3	2
21697	Sunbeam	USA	USA-HYB	3	4	2	2	4	3	3	1
21698	Bianca	USA	USA-HYB	4	4	1	3	5	3	3	1
56013	Cascade	USA	USA-HYB	3	4	2	2	1	1	3	3
60037	Wyoming 2-1	USA	USA-HYB	4	5	2	2	1	1	3	3
62013	Comet	USA	USA-HYB	2	5	2	1	1	3	2	2
65101	Talisman	USA	USA-HYB	4	5	2	3	2	2	1	3
65102	Cluster (L-1)	USA	USA-HYB	2	5	2	2	5	3	1	1
65104	Cluster (L-8)	USA	USA-HYB	2	5	2	1	1	3	2	3
		Subpool IG mode:		2	4	2	2	3	3	3	1
		Similarity index:		0.38	0.29	0.55	0.37	0.29	0.53	0.45	0.42
21039	Golden Star	Japan	A-HYB	1	3	1	2	3	2	3	2
21232	69K-BH66	Japan	A-HYB	4	4	1	3	5	2	3	2
21233	70K-SH6	Japan	A-HYB	3	1	1	2	5	3	1	1
21286	Kirin II	Japan	A-HYB	2	4	1	4	4	1	3	3
21676	Toyomidori	Japan	A-HYB	4	5	2	4	4	3	3	1
21677	Kitamidori	Japan	A-HYB	4	3	2	4	3	3	3	1
21678	Eastern Gold	Japan	A-HYB	4	4	2	4	5	3	1	2
60042	Shinshuwase	Japan	A-HYB	4	4	2	3	1	1	2	3
		Subpool IG mode:		4	4	1	4	5	3	3	2
		Similarity index:		0.50	0.42	0.56	0.44	0.36	0.44	0.53	0.42
21188	NP/55	BC	BC-HYB	2	4	1	4	5	3	3	1
21405	Super Alpha	BC	BC-HYB	1	2	1	4	4	3	3	1
21609	Pacific Gem	BC	BC-HYB	4	5	2	4	5	3	3	1
66054	Calicross	BC	BC-HYB	4	5	1	2	2	2	2	3
66050	First Choice	BC	BC-HYB	4	5	2	2	1	1	2	3
66056	Smoothcone	BC	BC-HYB	1	2	1	3	4	3	2	3
		Subpool IG mode:		4	5	1	2	4	3	3	1
		Similarity index:		0.39	0.39	0.64	0.46	0.46	0.39	0.50	0.57

† Descriptors: YLD, hop cone yield; HSI, hop storage index; CoH, cohumulone; M, myrcene; C, caryophylene; H, humulene.
‡ Genetic diversity pools were determined by cluster analysis of the normalized values for the eight hop quality descriptors.
§ Numbers in parentheses indicate the number of classes for each interpretive descriptor group.
¶ Similarity index is the percentage of possible interpretive group class comparisons in a genetic diversity pool that are the same.

Table 4. Relationship between England and Continental Europe subgroups within the European-cluster for eight quality characteristics for hops.

Quality characteristic	Subgroup: Continental		Significance level
	England (11)	Europe (15)	
			P†
Yield (kg ha^{-1})	478.5	357.5	0.21
α Acids (%)	5.2	4.1	0.02
β Acids (%)	2.6	2.9	0.27
HSI	0.3	0.3	0.25
CoH (%)	27.0	24.1	0.03
M (v/v)	38.9	37.6	0.74
C (v/v)	10.7	9.2	0.48
H (v/v)	26.6	24.7	0.46

† Means within rows are different at the significance level indicated on the basis of Fisher's Protected Least Significant Difference test.

British Commonwealth countries, utilize cultivars with higher levels of C. Yield differences among HYB subgroups are not representative of breeding quality as simply the difference in the environment that these cultivars were developed.

Our genetic diversity pool observations generally agree with research using DNA molecular markers. It is assumed that large numbers of categorizing variables result in a more precise classification. This assumes the descriptors used in the classification are not correlated with one another. In all cases of published work using DNA molecular markers as means of determining genetic relatedness, there was no discussion about the discriminatory value or contribution of individual molecular markers. It would be interesting to see how few molecular markers are actually required to determine the same classification as that observed with the full array of markers. We analyzed each hop quality descriptor for collinearity (Table 6). Each variable was subsequently omitted from the analysis and the resulting clusters observed for relationships to known pedigrees. Omitting one or more of the variables resulted in nonsensible classifications with unusual relationships between accessions that had little genetic relatedness based on known pedigree. Thus, all eight descriptor variables were necessary for reasonable clustering of accessions (data not shown).

Several noteworthy accession relationships were revealed by this study. Two full-sib sisters, 'Saxon' and 'Viking', were separated by 0.25 on a scale of 0 to 50.0. Three older Noble aroma cultivars, Lubelski, Spalter, and Saazer 36, were all three closely related (0.19–0.24) suggesting clonal relatedness. Using AFLP, Seefelder et al. (2000) could not identify any genetic differences between Spalter and Saazer. Similarly, four other accessions (Fuggle, 'Styrian', 'Savinja Golding' and 'USA Tettnang') all grouped together into a single cluster. The later three accessions are thought to be clonal selections from Fuggle. Both Sustar-Vozlic and Javornik (1999) and Jakse et al. (2001) could not differentiate genetic differences (on the basis of DNA) between Fuggle and Savinja Golding. Styrian is thought to be Fuggle introduced to former Yugoslavia circa 1900 (personal communication, A. Haunold, 1998). A fourth accession, 'Bramling', also grouped together with this cluster. Bramling is an old English cultivar from the 19th century and its origin is not entirely clear. Whether or not Bramling is a clonal selection from Fuggle is unknown. Finally, 'Saazer 36' and 'Tettnanger' grouped together in one cluster, which is not unexpected given the known genetic similarity of these two accessions (Seefelder et al., 2000).

Other observations merit mention. First, the two hybrid cultivars, Alliance and Progress, both developed

Table 5. Relationship between five geographic subgroups within the Hybrids-cluster for eight quality characteristics for hops.

Quality characteristic	Subgroup: UK	CU	USA	Japan	BC	Significance level
	n					*P*
	13	30	21	8	4	
Yield (kg ha^{-1})	881.5bc†	809c	1296.0a	1194.3ab	1120.3abc	0.01
α Acids (%)	9.5	7.3	9.2	8.3	9.3	0.10
β Acids (%)	4.5	4.6	4.8	5.0	4.8	0.79
HSI	0.27	0.25	0.26	0.25	0.24	0.07
CoH (%)	29.2bc	26.3c	36.6a	40.4a	35.4ab	0.01
M (v/v)	55.8	54.1	57.3	55.6	60.9	0.57
C (v/v)	8.7	7.6	9.4	8.5	14.0	0.07
H (v/v)	14.4	16.9	12.5	14.5	13.7	0.25

† Means within rows followed by a different letter are significantly different on the basis of Fisher's Protected Least Significant Difference test.

Table 6. Pearson correlation coefficients (*r*) for eight quality descriptors for the 129 hop accessions in the USDA-ARS collection.

Descriptor	α Acids	β Acids	HSI	CoH	M	C	H
				r			
Yield	0.57***	0.44***	−0.20	−0.03	0.39***	−0.20	−0.16
α Acids		0.51***	−0.30*	−0.26	0.40***	−0.21	−0.08
β Acids			−0.45***	−0.16	0.23***	−0.18	−0.14
Hop storage index (HSI)				0.31*	−0.17	0.14	−0.06
CoH					0.28*	0.37***	−0.65
M						−0.19	−0.59***
C							−0.17

* Indicates significance at $P \leq 0.05$ on the basis of the Bonferroni inequality adjustment.
*** Indicates significance at 0.001 on the basis of the Bonferroni inequality adjustment.

by Wye College (United Kingdom), were placed in the UK group as opposed to EU-HYB. Obviously, these two cultivars have similar traits to those observed for older, traditional UK cultivars. Second, the cultivar Tettnanger, originally obtained by the USDA-ARS in 1961, was ranked in the heirloom EU group while Tettnanger A and Tettnanger B, later acquisitions thought to be clonal selections from a Tettnanger field, were classified with the EU-HYB group. Similarly, the original acquisitions of Saazer was placed in the EU heirloom group while a subsequent acquisition of Saazer, Saazer 36 (also thought to be a clonal selection out of a Saazer yard), was ranked with the EU-HYB group. Certainly, the fact that fewer samples have been taken off the later acquisitions than the original ones suggests the possibility that the full range of phenotypic expression for the newer acquisitions has not been observed as of yet.

Germplasm maintenance, classification, and integration of new accessions require sufficient knowledge of the relationships among current members of the collection to determine their novelty. Without such knowledge, inclusion of redundant materials increases collection maintenance costs. The information contained in this report is the first such attempt at classifying phenotypic relationships amongst members of the U.S. hop collection, with the inclusion of wild North American accessions. Current work is underway classifying this collection by DNA makers, determining relationships of markers to hop quality phenotypic descriptors, and determining genetic × environmental interactions.

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